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INCIDENCE OF EPSTEIN-BARR VIRUS IN ASTRONAUT SALIVA DURING
SPACEFLIGHT

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Abstract

Payne DA, Mehta SK, Tying SK, Stowe RP, Pierson DL. Incidence of Epstein-Barr virus in astronaut saliva during spaceflight.

Background: Astronauts experience psychological and physical stresses that may result in reactivation of latent viruses during spaceflight, potentially increasing the risk of disease among crew members. **Hypothesis:** The shedding of Epstein-Barr virus (EBV) in the saliva of astronauts will increase during spaceflight. **Methods:** A total of 534 saliva specimens were collected from 11 EBV-seropositive astronauts before, during, and after four space shuttle missions. The presence of EBV DNA in saliva, assessed by polymerase chain reaction (PCR), was used to determine shedding patterns before, during, and after spaceflight. **Results:** EBV DNA was detected more frequently before flight than during ($p < 0.001$) or after ($p < 0.01$) flight. No significant difference between the in-flight and postflight periods was detected in the frequency of occurrence of EBV DNA. **Conclusions:** The increased frequency of shedding of EBV before flight suggests that stress levels may be greater before launch than during or after spaceflight.

Key words:

herpesvirus 4, human

stress

space flight

Latent virus infections are ubiquitous and Epstein-Barr virus (EBV), a DNA virus, infects over 90% of the population (9,13). EBV is highly infectious and can be transmitted by microdroplets and by direct contact with saliva. After the acute infectious phase, EBV can become latent in B lymphocytes. EBV is the causative agent of infectious mononucleosis and is associated with several malignancies, including Burkitt's lymphoma, nasopharyngeal carcinoma, and diffuse oligoclonal B-cell lymphoma (1,4,6,9,12,18). Latent EBV may be reactivated by a range of physical and psychosocial stress factors and shed in saliva (2,3). The immune system, specifically the cell-mediated immune (CMI) component, controls localized EBV infections following reactivation and prevents further systemic disease (16,20). Previous studies have demonstrated decreased CMI response during spaceflight (21,22). Similar decreases in CMI response have also been reported in the Australian Antarctic spaceflight analog (10,11).

Latent viruses pose an important infectious disease risk to astronauts during spaceflight, and this risk almost certainly increases as the duration of space missions increases. Risks associated with many infectious agents are reduced by restricting preflight contact of the flight crews with high-risk populations. However, latent viruses are unaffected by such precautions. This is the first study of latent viruses during spaceflight.

We used the polymerase chain reaction (PCR) to detect EBV DNA in saliva as a measure of viral shedding before, during, and after space flight. EBV DNA was most frequently detected in saliva specimens collected before flight.

Methods

Subjects

Subjects in this study were 11 astronauts (8 men, 3 women), all seropositive for EBV, from four space shuttle missions ranging in duration from 10 to 16 days. Baseline (preflight) shedding patterns were determined from saliva samples collected upon rising every other day for

two months, beginning about 6 months before launch. In-flight saliva samples were collected daily by each subject throughout the missions. Postflight samples were collected on landing day and then every other day for two weeks. The study was approved by the Johnson Space Center Institutional Review Board.

Samples

Samples were collected with Salivette kits (Sarstedt, Inc., Newton, NC), which consist of a cotton roll in a polypropylene vial. To collect a sample, a subject placed a roll in his or her mouth until it became saturated, and then returned the roll to the vial. Samples collected before and after flight were centrifuged immediately after collection and stored frozen at -70°C . Because frozen storage was not available during flight, crew members collected the samples in the same way as before launch, but added 1.0 ml of a biocidal storage buffer (1% sodium dodecyl sulfate, 10 mM tris(hydroxymethyl)aminomethane-Cl, and 1mM ethylenediaminetetraacetic acid) to the vial after the saturated roll had been placed in it. These vials then were stored at room temperature during the mission. When the vials were returned to the laboratory, the saliva was collected by centrifugation and stored frozen until analysis. All samples collected from each mission were analyzed simultaneously. Ground-based analysis verified that the stability buffer preserved the viral DNA for subsequent polymerase chain reaction (PCR) analysis with no observable differences from the pre- and postflight treatment procedure.

Saliva specimens were concentrated with a Microsep 100K filtration unit (Filtron Technology Corporation, Northborough, MA) and extracted by a nonorganic extraction method (Qiagen Inc., Chatsworth, CA). Microcarrier gel (Molecular Research Company) was added to facilitate DNA recovery at the proteinase K digestion step (Boehringer Mannheim, Indianapolis, IN). DNA was resuspended in 50 μl of nuclease-free water (Amresco, Solon, OH). EBV DNA for control studies was obtained from Sigma Chemical Co. (St. Louis, MO).

Molecular Studies

PCR primers directed at the EBV polymerase accessory protein gene (BMRF1) were P1, 5',3'-GTCCAAGAGCCACCACACCTG (The Midland Certified Reagent Co., Midland, TX), and P2, 5',3'-biotin CCCAGAAGTATACGTGGTGACGTAGA (Digene Diagnostics, Gaithersburg, MD) (17). These primers were used at a concentration of 200 μ M with 10 μ M deoxynucleic acid triphosphates (Perkin-Elmer, Branchburg, NJ). PCR was optimized using buffer II (Perkin-Elmer) with 2.5 mM MgCl₂ (14). Dimethyl sulfoxide (Sigma) was added to a final concentration of 5% (15). AmpliGold (2.5 units) (Perkin-Elmer) was added per 100 μ l reaction mixture; 5 μ l of the purified DNA was added to 20 μ l of the reaction mixture. The cycle parameters were 95° C for 9 minutes, followed by 40 cycles of 94° C for 15 seconds, 61° C for 15 seconds, and 72° C for 15 seconds, with a final extension step at 72° C for 5 minutes. PCR fragments were detected with the PCR Sharp System (Digene Diagnostics) after 24 hours.

Data Analysis

Statistically significant differences between sample periods were assessed with analysis of variance (ANOVA) and the Chi-square test.

Results

A total of 534 saliva samples were collected from 11 crew members on four space shuttle missions to determine the effect of spaceflight on shedding patterns of EBV. EBV DNA was detected in 18% (51/281) of the preflight specimens, 9% (13/140) of the in-flight samples, and 6% (6/113) of the postflight samples. Each subject collected an average of 48.5 samples; the average number of specimens positive for EBV DNA per crew member was 6. Two astronauts had 17 or more specimens with detectable EBV DNA. Eight astronauts had 6 or fewer positive samples, and one had no detectable EBV in the saliva during the entire collection time. Ten crew members shed EBV DNA during the 60-day preflight collection period. Only two crewmembers shed EBV DNA during flight and three crewmembers shed after landing. Chi-square results confirmed that viral shedding was higher before launch than either during ($p < 0.001$) or after ($p < 0.01$) flight (Figure 1). Similar results were observed with analysis of variance. The frequency of

EBV DNA appearance was the same during the in-flight and postflight periods, as confirmed with the general linear models procedure.

[Figure 1 Here]

[Figure 2 Here]

Figure 2 illustrates the occurrence of EBV DNA in one crew member's saliva samples before, during, and after flight. EBV DNA was detected throughout the preflight collection period, but was present less frequently during and after flight. EBV DNA was detected in the first four in-flight saliva samples (flight days 2–5), but was not present during the rest of the flight or after landing. The only other crew member to shed EBV DNA during spaceflight had all but two samples positive throughout the in-flight period.

Discussion

This is the first report on the reactivation and shedding of a latent virus during spaceflight. The high prevalence of EBV in the general population and the ease with which saliva specimens can be collected during spaceflight make EBV an ideal indicator for assessing the effect of spaceflight on viral shedding in astronauts. Unique aspects of spaceflight, such as the ease of generation of aerosols from liquids such as saliva or urine, increase the risk of contact with infectious particles. Microgravity may allow exposure to larger droplets containing larger numbers of viruses.

The observed differences in the presence of EBV DNA in astronauts' saliva before, during, and after spaceflight may reflect varying levels of physical or psychosocial stress at those times, and perhaps varying levels of stress hormones (3,5,7,8,19). EBV in lymphoblastoid cells can be reactivated in the presence of hydrocortisone, dexamethasone, corticotropin-releasing factor, adrenocorticotrophic hormone, or somatostatin (3). Stress hormones were not measured

on the missions included in the current study, but indications of increased stress associated with spaceflight are well documented (5,19).

The reactivation of latent viruses is commonly associated with a preceding decrease in the CMI response (16,20). Shedding of latent viruses may prove to be an early indicator of a compromised CMI response. This could be a valuable tool in detecting asymptomatic changes in the immune system, allowing early intervention to prevent or limit clinical symptoms.

The viral shedding patterns found in this study appear to disprove the initial hypothesis that viral shedding is greater during the flight phase, suggesting that stress capable of reactivating viruses is greatest before spaceflight. These results contrast with similar studies during the winter-over period at the Australian Antarctic stations and during 60- and 90-day closed environmental chamber studies (unpublished data). In both of these ground-based analogs of spaceflight, viral shedding was greatest during the period of isolation. Decreased CMI has been well established at the Antarctic stations during physical isolation (11,23), and decreases have more recently been observed during isolation in the environmental chambers (C. Sams, written communication).

Future studies on upcoming space shuttle missions include quantifying the copy number of EBV, determination of infectivity of virus in saliva, and expanding the study to include other latent viruses in saliva and urine.

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Figure Legends

Figure 1. EBV DNA detection in saliva from 11 seropositive astronauts before, during, and after flight.

Figure 2. EBV DNA detection in saliva of a crewmember before, during and after a 12-dayspaceflight.

Figure 1 D. Payne et al. Top

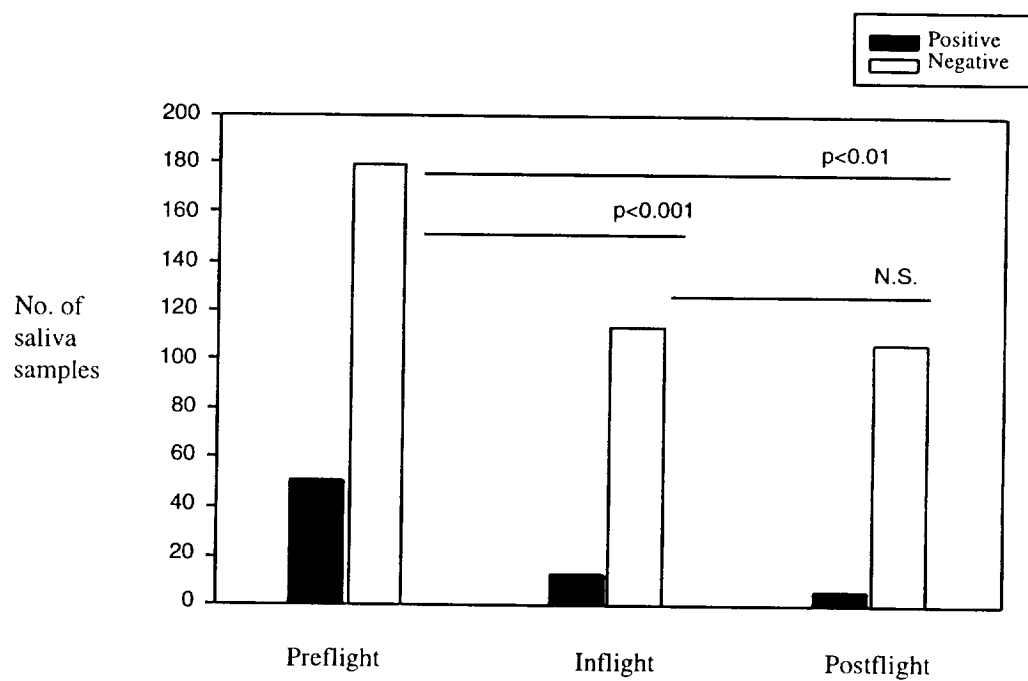


Figure 2 D. Payne et al. Top

